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Flow Injection Calibration Techniques

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Anwendung der Fließinjektions-Technik für Eichmethoden

Summary. The role of calibration in the overall analytical procedure is discussed and the potential role of flow injection techniques are assessed. The factors affecting the dispersion obtained in a flow injection system are described and examples given of how control of the various factors can be exploited to produce manifolds for calibration purposes. Most of these examples concern atomic spectrometry methods for which there is considerable interest at present in developing extended range calibrations and flow injection versions of the standard additions method. Several examples of the latter are given.

Introduction

The aim of an analytical method is to provide information about the chemical composition of a bulk material on which to base decisions that govern a future course of action. The analytical method is made up of several stages including sampling, pretreatment, measurement, conversion of instrument response to chemical information and evaluation of the quality of the information. The stages in a particular method will be selected, after dialogue with the end-user of the information, to take account of all the constraints imposed by the problem. For instrumental quantitative analyses, the conversion of instrument response to chemical information is made by calibrating the off-null registration of the instrument used, usually at the time the analysis is performed. This procedure typically involves preparation of a stock concentrated solution of the analyte of known concentration, serial dilution using calibrated glassware to produce a set of standard solutions whose concentrations cover the instrument's working range, addition of reagents as appropriate, measurement of the desired property and finally fitting a curve to the calibration data points. There are several variations on this basic calibration theme in common use. For example, the calibration strategy may be designed to off-set interference effects from other matrix components and this could be done either by matrix matching or by standard additions.

Calibration is necessary because, for many commonly used analytical chemistry instruments, the relationship between instrument response and amount of analyte species is either not known accurately or is only known accurately for particularly simple matrices. In addition the response of some instruments, such as those for performing flame atomic absorption spectrometry (FAAS) may be heavily dependent on the operating conditions selected as well as the particular design (and age) of instrument used. The calibration procedure affects the speed, accuracy and precision of an analytical procedure. The preparation and measurement of standards can occupy a considerable proportion of the working time. If the standards chosen are inappropriate for the analyte/matrix combination being examined, inaccurate results will be obtained and the uncertainties of the curve fitting procedure will contribute to the overall 'plus or minus' term associated with the final result.

Flow injection techniques

Developments in analytical methodology occur because the proposed alternative new methods offer improvements in terms of the criteria used to evaluate method performance. Flow injection (FI) techniques [1] offer improvements in most of the stages of an analytical method, particularly pretreatment and measurement. All steps involved in analytical measurement may benefit from the use of FI techniques including sample introduction and calibration, not just in terms of doing what went before better, but also by introducing a completely new set of pretreatment and measurement strategies.

This brief review is concerned with the use of FI techniques for calibration strategies. The various strategies that have been devised will be illustrated by examples concerned mainly with atomic spectroscopy techniques, for which there is a rapid growth of FI applications [2, 3].

Dispersion

One of the fundamental characteristics of flow injection methodology is that controlled, precise dispersion is obtained. It is this feature which is the basis for the use of FI techniques for calibration purposes as an appropriately designed manifold provides the alternative to calibrated glassware for dilution purposes. It should be borne in mind that any technique which is designed to dilute a stock standard by several factors, to produce a set of calibration standards, may be applied for the dilution of off-range samples.

The factors which control dispersion are flow rate, volume injected, tube dimensions, manifold design (confluences, splits, coiling etc.) together with detector characteristics and injection method. As peak height at the maximum is readily measured, particularly if a recording is made of

the transient response, this is normally used as the analytical parameter. Furthermore the extent of the dispersion produced by a particular apparatus is usually quantified by the factor by which the injected solution has been diluted at the peak maximum. This dilution factor is referred to as the dispersion coefficient, D, and a set of guidelines have been established relating changes in D to changes in operating parameters [1].

In some cases variation of D is achieved by controlled timing of a particular operation. The volume injected may be varied by alterations of the injection time during which the partial contents of the sample loop are introduced into the system. Alternatively, a second injection valve may isolate a small segment of an already dispersed zone (the zone sampling method). Readings may be made on the peak profile at times other than that corresponding to the peak maximum and peak width may also be used as a quantitative parameter.

All of these time based methods together with methods based on flow rate control, tube dimensions, networking (split and confluence) and merging zones will be described.

Manifold design 1. Merging zones

The merging zone manifold is shown in Fig. 1. Zagatto et al. [4] used a sample line flow rate of 1 ml min^{-1} and a reagent line flow rate of 12 ml min^{-1} to both add lanthanum and achieve an overall D value of 40 in the determination of Ca, K and Mg in plant digests by FAAS. To maintain the flow to the nebuliser at 7 ml min^{-1}, the flow was split just prior to the detector. A precision of 0.5% RSD was reported and the method consumed only 500 µg of La per sample. Thus, in addition to dilution of off-range samples, a number of other benefits from the use of the FI-FAAS combination are obtained for this particular analysis.

Asynchronous merging of reagent and sample was used by Mindegard [5] to determine serum albumin by solution spectrophotometry. The reagent zone was timed to overlap an appropriate portion of the tail of the dispersed sample zone and a calibration range of 0–60 g l^{-1} was obtained.

Manifold design 2. Variable tube dimensions

By switching the same injected volume (12.5 µl) along tubes of different dimensions (length and internal diameter) dispersion coefficients ranging from 6 to 40 in six discrete steps were obtained [6]. The manifold is shown in Fig. 2 and it was applied for the determination of Ni, Ca and Cr by FAAS. Solutions of concentration 300, 100 and 250 mg l^{-1} respectively were used for calibration purposes and recoveries of 100 ± 2% were obtained.

Manifold design 3. Networks

A network manifold is shown in Fig. 3a. This was used to produce 5 calibrations of varying sensitivity for the determination of Mg by FAAS [7]. The range covered was 0–10 mg l^{-1}. As the connecting lines between the split and confluence points are of different dimensions, three unequal asynchronously merged peaks are obtained as shown in Fig. 3b. The absorbance values of the 3 peaks and 2 troughs are used as the calibration points.

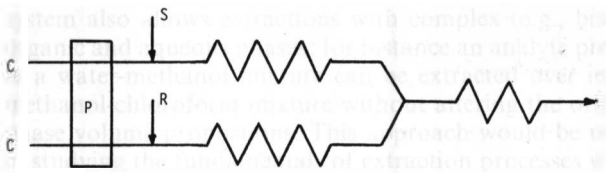

Fig. 1. Merging zone manifold. *S* sample injection, *R* reagent injection, *C* carrier streams, *P* peristaltic pump. After merging the overlapping zones are transported to the FAAS detector

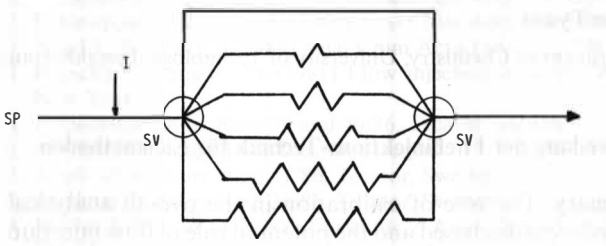

Fig. 2. Variable tube dimensions manifold. *SP* syringe pump, *I* injection valve, *SV* 6-way switching valve. The same standard is injected 6 times and switched down each of the 6 lines of different dimensions in turn

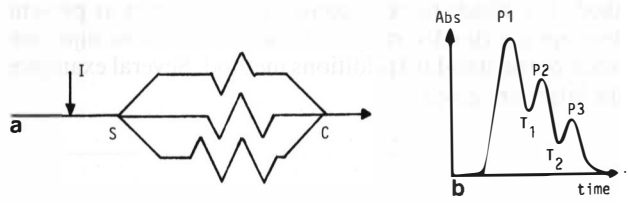

Fig. 3a, b. **a** Network manifold. *I* injection point, *S* 3-way split, *C* confluence point. **b** Absorbance-time profile produced. Each injection produces 5 calibration values, 3 peaks (*P*1, *P*2, *P*3) and 2 troughs (T_1, T_2).

Time based methods 1. Variable injection volume

For many manifold designs it has been observed that there is an exponential relationship between dispersion coefficient and volume injected, V_i, of the form

$$D = [1 - \exp(-V_i k)]^{-1}$$

where k is a constant[1] for a given set of experimental conditions. Thus variation of the volume injected can be a powerful way of changing D. For a system using an injection valve, controlled timing may be used either to inject part of the loop contents or to reinject part of the dispersed zone by means of a second valve. A manifold for the latter procedure (known as zone sampling) is shown in Fig. 4. The system was used by Reis et al. to determine K in plant digest over the range 0–500 mg l^{-1} by FAAS (D up to 130) with a precision of about 1% [9]. Sherwood et al. [10] have developed a system using controlled dispersion based on controlled timing of a pump and probe. The manifold is shown in Fig. 5. Initially the probe rests in a reagent (20×10^{-3} M EDTA) carrier solution and the pump is running. The pump is stopped, the probe transferred to the sample solution and the pump restarted for a controlled period. The pump is

[1] This relationship is also that predicted by the single, well-stirred tank model for dispersion behaviour [9], in which case k is the reciprocal of the tank volume

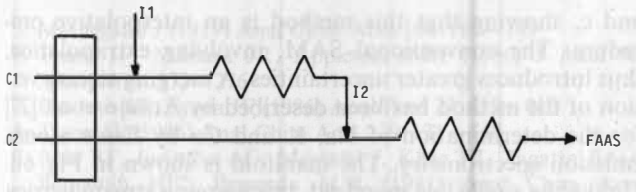

Fig. 4. Zone sampling manifold. *I*1 first injection point, *I*2 second injection point at which a portion of the disperison zone from *I*1 is injected into carrier *C*2 and transported to the flame atomic absorption spectrometer, FAAS

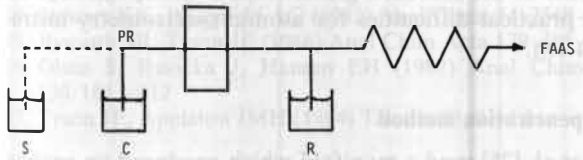

Fig. 5. Pump and probe manifold. *PU* computer-controlled, stepper motor driven pump, *PR* computer-controlled moveable probe, *C* carrier reagent solution *S* sample solution, *R* reservoir of carrier solution. The *dotted line* shows the position of the probe during sampling. The pump is stopped during probe transfer. To avoid nebuliser running dry during pump stop periods, reagent is aspirated from reservoir *R*

again stopped, the probe returned to the reagent solution and the pump restarted. Solution continues to flow to the instrument during the pump-stop periods as the negative pressure developed at the nebuliser of the FAA instrument draws solution from the reservoir. The manifold was used for determining Mg in clinical samples (whose salty residues can cause problems of wear for rotary valves) at *D* values up to 80. It was found that a suitable calibration for Mg could be obtained by aspirating 1.25, 2.50, 3.25 and 5.00 µl of a 1×10^{-3} mol/l Mg solution.

Time based methods 2. Peak width

Several workers have shown that the width of an exponential peak is related to a logarithmic function of the concentration of the injected solution [8, 11, 12]. If the manifold dispersion behaviour is modelled by passage of a slug of volume V_i through a tank of volume V at flow rate u, the width of the peak, Δt, at concentration C' is given by

$$\Delta t = (V/u) \ln[(C_m/C') - 1] - (V/u) \ln(D - 1)$$

where C_m is the injected concentration.

Good agreement with the form of this equation was obtained for the determination of Mg over the range 1 – 1000 mg l^{-1} by assuming that the nebuliser of the FAA spectrometer behaved as a well-stirred mixing chamber [13]. Stewart and Rosenfeld incorporated a real mixing chamber into the manifold and demonstrated extended range calibrations for solution spectrophotometry, conductimetry, flame atomic emission spectrometry and molecular fluorescence spectrometry [14]. It has been shown [8] that the approximate equation (based on the assumption $C_m/C' \gg 1$)

$$\Delta t = (V/u) \ln C_m - (V/u) \ln C'(D - 1)$$

may be used with little loss in linearity, and the use of this equation for extended range calibration for Mg by FAAS has been demonstrated [7]. Errors of 0.0, −5.0, 0.0 and + 3.0 were obtained at 3, 50, 400 and 1000 mg l^{-1}, respectively. It has also been shown that rather than construct a calibration curve by injection of a variety of solutions with different C_m values and measuring Δt at the same value of C' for each peak, it is possible to obtain calibration data from a single peak by measuring Δt at a variety of C' values [15]. Larger errors were obtained with this latter method and it was suggested that this method might only be suitable as a rapid screening method enabling a dilution factor to be calculated to allow measurement by a more accurate calibration procedure.

Time based methods 3. Continuous dilution

Two variations of this method have been described. In the first, Olsen et al. [16] use data taken from the fall curve of a normal flow injection peak at known times after the instant of injection. Previous experiments have established how concentration is related to the delay time used and thus a calibration may be constructed by measuring the instrument response (in this case solution absorbance) at a set of known delay times. In the second method, Tyson and Appleton [17] used a real mixing chamber to generate an exponentially increasing concentration gradient by continuous dilution of a concentrated standard. The concentration, C, time, t, profile has the equation $C_m = C\,[1\text{-}\exp(-ut/V)]$ where C_m is the standard concentration. During the calibration step, the tank effluent is fed directly to the nebuliser of the FAA spectrometer and the instrument's absorbance-time profile recorded with a microcomputer. The absorbance of the unknown is compared with this stored data and a characteristic time value obtained which can be substituted into the above equation. As all the terms on the right hand side of the equation are known, a value of C may be calculated. Accuracies of within ±3% were obtained for Mg, Ni and Cr. A similar method, based on the exponential decay curve for the wash-out of a well stirred tank, has been used by Horvai et al. [18] to calibrate ion-selective electrodes and to determine selectivity coefficients.

Standard additions method

At least five flow injection methods have been proposed for calibration by the standard additions method (SAM). Some of the manifolds involved are illustrated in Fig. 6.

Merging zones method

The simplest form of the merging zones SAM manifold is shown in Fig. 1. This was used by Zagatto et al. [19] for the determination of Cu, Ni and Zn by the so-called "generalised SAM", which compensates for both matrix and spectral interferences, in conjunction with inductively coupled plasma atomic emission spectrometry (ICP-AES). In order to vary the concentration of the standard, a zone sampling manifold (Fig. 4) was combined with the merging zones principle by Gine et al. [20]. The appropriate part of the manifold is shown in Fig. 6a. The analysis involved was the determination of nitrate in plant extracts by solution spectrophotometry.

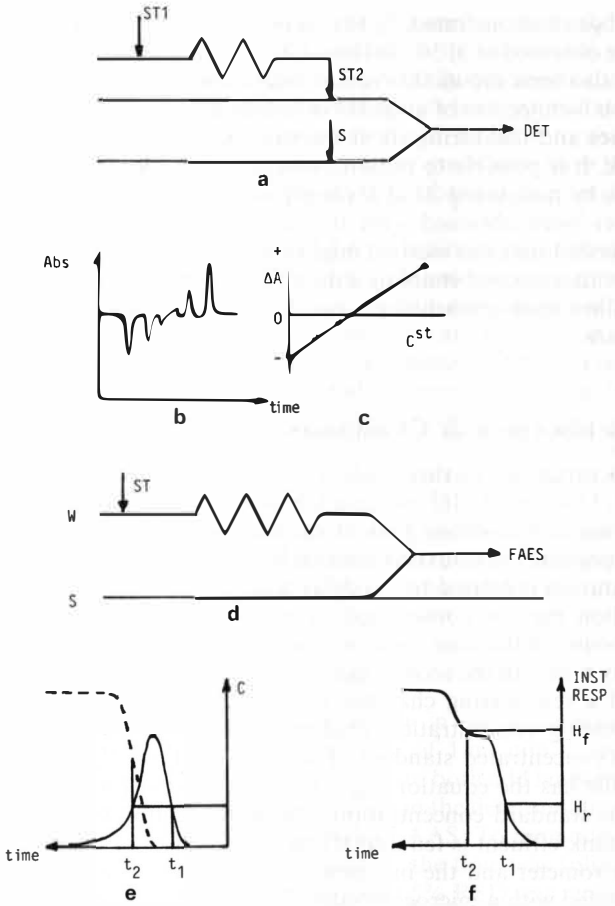

Fig. 6a–f. Standard addition manifolds. **a** Zone sampling and merging zones manifold. The standard is injected at *ST*1 and at an appropriate time (which can be varied), a portion is injected into the merging zones part at *ST*2. Injection of the standard at *S* is synchronised and after merging the overlapping zones are transported to the detector, *DET*. **b** Single line reverse method. Standards of increasing concentration are injected into the sample used as carrier stream. **c** Plot of ΔA against the concentration of standard, C^{st}. When $A = O$, the concentration is that of the sample. **d** Merging stream manifold. Standard is injected at *ST* into a water carrier stream *W* and merged with the sample stream *S*. The overlapping zones are then transported to the flame atomic emission spectrometer, FAES. **e** Zone penetration method. *Solid curve* shows dispersed sample zone, *dotted curve* shows dispersed edge of standard zone. **f** The instrument response to the situation shown in **e**. H_r response (on *rise curve*) at t_1, H_f response (on *fall curve*) at t_2. H_r is due to the sample only whereas H_f is due to the same concentration of sample plus a concentration of the standard

Reverse FIA methods

In this technique the sample is used as the carrier stream and standards are injected. In the simplest version a single line manifold is used. The method was first described by Tyson [21] and has been used for the determination of Ca in iron ore [22], Cr in steel [23], Ca in cement [24], Si in phosphoric acid [25] and Ba and Cr in river sediment and coal fly ash [26]. The last three examples are for ICP-AES of which the last two use a simplified version of Tyson's original method based on injection of a blank and a standard of concentration greater than that sought. A schematic diagram of the instrument response as a function of time and the corresponding calibration plot are shown in Fig. 6b and c, showing that this method is an interpolative procedure. The conventional SAM, involving extrapolation, thus introduces greater uncertainties. A merging stream version of the method has been described by Araujo et al. [27] for the determination of Na, K and Ca by flame atomic emission spectrometry. The manifold is shown in Fig. 6d. Unlike the single line manifold, this merging stream version requires an extrapolative calibration procedure. A similar idea has been proposed by Greenfield [24]. This method suggested the use of a well-stirred tank to produce an exponential gradient of added standard concentration in the merging stream. The method was based on a step charge in flow rate as the standard gradient was added, which could cause practical difficulties for atomic spectrometry instrumentation.

Zone penetration method

Fang et al. [28] used a manifold which produced an analyte solution zone sandwiched between water (in front) and standard (at the rear). The overlapping zones are shown in Fig. 6e and the net result on the signal in Fig. 6f. The points t_1 and t_2 on the rise and fall curves, respectively, correspond to the same dispersion coefficient and as the system behaves as a single line manifold, it is possible to calculate the concentration of the standard at a point of known dispersion coefficient of the sample [8]. The concentration of sample, C^s, may be calculated from the equation $C^{st} = C^s(R_H - 1)/D - 1$ where C^{st} is the concentration of standard, R_H is the ratio of instrument responses at t_2 to that at t_1. It is not necessary to conduct a separate experiment to establish D as this may be obtained from the equation $\Delta H = kC^{st} - k/D$ where ΔH is the difference in instrument responses at t_2 and t_1 and k is the slope of the conventional calibration plot. The method was, in fact, used with a single standard and thus D values had been previously established. Calcium was determined at the 47 mg l^{-1} level in a soil extract using an 80 mg l^{-1} standard.

Conclusion

Considerable ingenuity has already been shown in devising flow injection methods for the on-line production of solutions for calibration purposes. In addition to the adaption of existing techniques for calibration, flow injection techniques offer a number of entirely new calibration strategies such as peak width methods, the interpolative reverse FIA standard additions method and continuous dilution methods. It seems unlikely that all the ways of extracting analytical information from the precise concentration gradients produced by flow injection methods have been discovered and thus develoments in the use of flow injection methodology for calibration purposes will continue to be reported for some time to come.

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